

SELECTED  
JAN 4 1993

## EASTERN EQUINE ENCEPHALOMYELITIS VIRUS AND *CULISETA MELANURA* ACTIVITY AT THE PATUXENT WILDLIFE RESEARCH CENTER, 1985-90

BENEDICT B. PAGAC,<sup>1</sup> MICHAEL J. TURELL<sup>2</sup> AND GLENN H. OLSEN<sup>3</sup>

**ABSTRACT.** Mosquito population densities, virus isolations and seroconversion in sentinel quail were used to monitor eastern equine encephalomyelitis virus (EEE) activity at the Patuxent Wildlife Research Center, Laurel, Maryland, from 1985 through 1990. A dramatic increase in the number of *Culiseta melanura* collected in 1989, as compared with the 3 previous years, was associated with virus isolations from this species (5/75 pools;  $n = 542$  mosquitoes) and with seroconversion in sentinel quail (4/22 birds positive). This was the first detection of EEE virus activity in this area since a 1984 EEE outbreak killed 7 whooping cranes.

During 1984, an outbreak of eastern equine encephalomyelitis (EEE) virus occurred among birds at the Patuxent Wildlife Research Center (PWRC), in Laurel, MD (Dein et al. 1986; Carpenter et al. 1987, 1989). Of 39 whooping cranes [*Grus americana* (Linn.)] at the PWRC, 21 (54%) had evidence of infection with EEE virus, and 7 (33%) of the infected birds died (Dein et al. 1986). These deaths were of particular concern because the whooping crane is an endangered species, and the PWRC is one of 2 research facilities devoted to the propagation of this species.

In the eastern United States, EEE virus is transmitted among birds by the mosquito *Culiseta melanura* (Coq.), and the virus can cause severe disease or death in horses, game birds and humans (Morris 1989). Mosquito surveillance at PWRC was initiated in late 1984, but no adult *Cs. melanura* were collected that year because trapping occurred after the first frost (Dein et al. 1986, Carpenter et al. 1987). Furthermore, EEE virus was not detected from over 900 *Cs. melanura* collected in the vicinity of PWRC in 1985 (Scott et al. 1987).

Because of the potential impact of EEE virus on the whooping crane recovery program, the risk of human and equine disease and the proximity of PWRC to Fort George G. Meade, MD, mosquito surveillance was conducted annually at PWRC from 1985 to 1990 to monitor mosquito population densities and virus activity.

Mosquitoes were collected with solid-state army miniature (SSAM) light traps (John W. Hock Co., Gainesville, FL) augmented with dry

ice. In 1985, four SSAM trapping sites were established along the Big Patuxent River flood plain, within a 3.2 km radius of the crane pens. These consisted of 2 pairs of sites separated by 3,000 meters. Within each pair, trapping locations were approximately 700 m apart. These 4 sites were used from 1985-90, and trapping methods remained the same throughout this period. Mosquitoes were trapped 2 nights per week. Trap indices [number *Cs. melanura* collected/(number of traps  $\times$  number of nights)] were calculated each week from August through September of each year.

Female mosquitoes were identified to species and counted from 1985 to 1990, but only assayed for virus in 1985, 1989 and 1990. Mosquitoes for viral assay were frozen at  $-70^{\circ}\text{C}$  in pools of  $\leq 25$  specimens of each species until tested. Pools of mosquitoes were triturated in 2 ml of diluent (2% fetal bovine serum in Hanks' balanced salt solution with HEPES buffer, antibiotics and  $\text{NaHCO}_3$ ) and tested for infectious virus by plaque assay on Vero cell monolayers. Seed stocks were made from all positive suspensions and the identity of viruses recovered was confirmed by a plaque-reduction neutralization test (Earley et al. 1967) with antisera directed against the prototype strains of EEE, Highlands J (HJ) and Jamestown Canyon (JC) viruses.

Virus transmission to avian hosts was monitored with sentinel bobwhite quail [*Colinus virginianus* (Linn.)]. Five to 7 seronegative quail were placed in wire cages in the vicinity of each of the four mosquito traps and bled monthly from September through November. Sera obtained from these birds were tested for neutralizing antibody to EEE virus (Earley et al. 1967).

*Culiseta melanura* populations generally remained low (mean = 2.9 mosquitoes/trap night) from 1986 through 1988 (Fig. 1). However, a significantly (2-way ANOVA test,  $P < 0.001$ ) greater number of *Cs. melanura* were collected per trap night during the 1989 trapping season, with a peak trap index of 28.4 in the third week of September. In contrast, the weekly trap in-

<sup>1</sup> U.S. Army Environmental Hygiene Activity-North, Entomological Sciences Division, Fort George G. Meade, MD 20755.

<sup>2</sup> Department of Epidemiology, Disease Assessment Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702.

<sup>3</sup> Patuxent Wildlife Research Center, U.S. Fish and Wildlife Service, Laurel, MD 20708.

DISTRIBUTION STATEMENT A

Approved for public release  
Distribution Unlimited

92-33157

AD-259 555

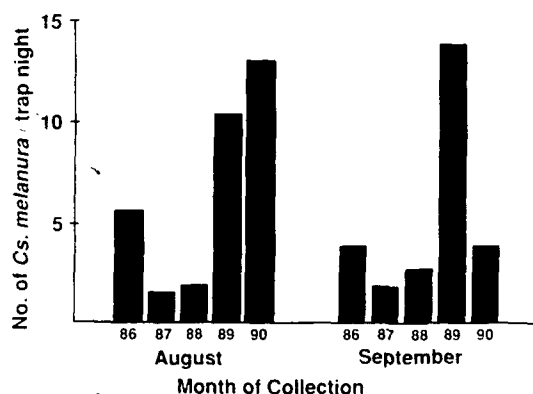


Fig. 1. Mean numbers of female *Culiseta melanura* collected per trap night in dry ice-augmented solid-state army miniature light traps are shown by month of collection for 1986 through 1990.

dices for *Cs. melanura* never exceeded 10 in the 3 previous years.

A total of 452 *Cs. melanura*, collected between September 11 and November 14, 1989, were tested in 75 pools; five pools (1% of the *Cs. melanura*) yielded EEE virus isolates (Table 1). Virus was recovered from mosquitoes collected on September 11, 18, 25 (2 isolates), and November 7. In addition, 117 *Coquillettidia perturbans* Walker were collected between September 11 and 25, 1989. These were pooled ( $\leq 10$ /pool) and assayed as described above. One isolate of JC virus was obtained from a pool ( $n = 10$ ) collected on September 11, 1989. All viruses were reisolated from the original mosquito suspensions and their identity confirmed by a plaque-neutralization test.

Comparatively large numbers of *Cs. melanura* (mean trap index = 8.9) were again collected in 1990, but, as occurred after the 1984 EEE outbreak, there was no evidence of EEE virus activity during the year following an EEE outbreak.

Four virus isolates (all HJ virus, a subtype of western equine encephalomyelitis virus), however, were obtained from 3 pools of *Cs. melanura* (447 tested) and one pool of *Culex erraticus* (Dyar and Knab) (310 tested). No virus was recovered from any of 820 *Cq. perturbans* tested.

Similar to the pattern observed in mosquitoes, no EEE virus activity was detected from 1985 through 1988 in sentinel quail (Table 1). Although half of the sentinel quail cages were destroyed by vandals before complete results could be obtained for 1989, neutralizing antibody to EEE virus was first detected in sera obtained from 4 (18%) of the 22 quail on 26 September. One quail had a titer of 1:16 on September 26, but died before the next bleed. When rebled on November 15, sera from the 3 surviving seropositive quail neutralized 80% of the plaques at dilutions of 1:64 ( $n = 1$ ) and 1:128 ( $n = 2$ ). None of the 22 sentinel quail in 1990 seroconverted for EEE virus.

In summary, we detected EEE virus activity in 1989 in the vicinity of the PWRC, both by isolating virus from field-collected *Cs. melanura* and by noting seroconversion in sentinel quail. This was the first documentation of EEE virus activity in this area since the 1984 epizootic that killed 7 whooping cranes. The proximity of the current outbreak to the whooping crane breeding facility raised concern about possible infection of this species and provided an opportunity to examine the efficacy of the EEE virus vaccine which is being administered to these birds (Clark et al. 1987). Currently, a study is in progress to evaluate the EEE viral exposure of whooping cranes that occurred during the 1989 EEE outbreak.

Also, in the years after both the 1984 and 1989 EEE virus outbreaks, EEE virus activity was not detected, despite relatively large populations of *Cs. melanura* present during each of these

Table 1. Surveillance for eastern equine encephalomyelitis virus activity at the Patuxent Wildlife Research Center from 1985 through 1990.

Year	Sentinel quail	<i>Culiseta melanura</i>		
	No. (% positive)	Trap index <sup>1</sup>	No. tested <sup>2</sup>	No. positive <sup>3</sup>
1985	25 (0%)	10.5 (64)	888 (116)	0
1986	20 (0%)	4.8 (72)	Not tested	Not tested
1987	20 (0%)	1.6 (72)	Not tested	Not tested
1988	20 (0%)	2.2 (72)	Not tested	Not tested
1989	22 (18%) <sup>4</sup>	11.7 (72)	452 (75)	5 (1:90)
1990	22 (0%)	8.9 (72)	447 (70)	0

<sup>1</sup> Trap index = number of *Cs. melanura* collected during August and September/no. trap nights (number trap nights).

<sup>2</sup> Number of *Cs. melanura* tested (no. of pools tested).

<sup>3</sup> Number of isolations (minimum field-infection rate).

<sup>4</sup> Some of the cages holding sentinel quail were destroyed by vandals after the September 26, 1989 bleed, thus the 18% represents a minimal infection rate.

years. One possible explanation for the lack of detectable EEE activity during the year after each major outbreak is that widespread immunity, developed in the avian population during each of the epizootics, prevented or hindered a renewed outbreak in the subsequent year.

We thank R. Tammariello for his technical assistance in conducting the various virus assays; M. Brosnihan, S. Doran, C. Ellison, H. Harlan, C. Hildebrandt, J. Holzinger, J. Jackson, K. Ratdke, P. Ross, P. Sandridge and R. Scott for collecting and processing mosquito samples; and S. Maltese for coordinating the quail studies.

Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the "Guide for the Care and Use of Laboratory Animals," NIH publication 86-23, 1985 edition. The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

#### REFERENCES CITED

- Carpenter, J. W., G. G. Clark and D. M. Watts. 1989. The impact of eastern equine encephalitis virus on efforts to recover the endangered whooping crane. pp. 115-120. In: International Council for Bird Preservation Technical Publication No. 10, Cambridge, England.
- Carpenter, J. W., F. J. Dein and G. G. Clark. 1987. An outbreak of eastern equine encephalitis virus in captive whooping cranes. pp. 123-127. In: J. C. Lewis (ed.), Proceedings of the 1985 Crane Workshop. Platte River Whooping Crane Habitat Maintenance Trust and U. S. Fish and Wildlife Service, Grand Island, NE.
- Clark, G. G., F. J. Dein, C. L. Crabbs, J. W. Carpenter and D. M. Watts. 1987. Antibody response of sandhill and whooping cranes to an eastern equine encephalitis virus vaccine. *J. Wildl. Dis.* 23:539-544.
- Dein, F. J., J. W. Carpenter, G. G. Clark, R. J. Montali, C. L. Crabbs, T. F. Tsai and D. E. Docherty. 1986. Mortality of captive whooping cranes caused by eastern equine encephalitis virus. *J. Am. Vet. Med. Assoc.* 189:1006-1010.
- Earley E., P. H. Peralta and K. M. Johnson. 1967. A plaque neutralization method for arboviruses. *Proc. Soc. Exp. Biol. Med.* 125:741-747.
- Morris, C. D. 1989. Eastern equine encephalomyelitis. pp. 1-20. In: T. P. Monath (ed.), The arboviruses: epidemiology and ecology, vol. 3. CRC, Boca Raton, FL.
- Scott, T. W., J. G. Olsen, T. E. Lewis, J. W. Carpenter, L. H. Lorenz, L. A. Lembeck, S. R. Joseph and B. B. Pagac. 1987. A prospective field evaluation of an enzyme immunoassay: detection of eastern equine encephalomyelitis virus antigen in pools of *Culiseta melanura*. *J. Am. Mosq. Control Assoc.* 3:412-417.

Carpenter, J. W., G. G. Clark and D. M. Watts. 1989. The impact of eastern equine encephalitis virus on

DTIC QUALITY INSPECTED 1

Accession For	
NTIS GRA&I	<input checked="" type="checkbox"/>
DTIC TAB	<input type="checkbox"/>
Unannounced	<input type="checkbox"/>
Justification	
By	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A-1	20